Supplementary Note

Synthesis and Characterization of Chemical Compounds

Thiolutin is a zinc chelator that inhibits the Rpn11 and other JAMM metalloproteases

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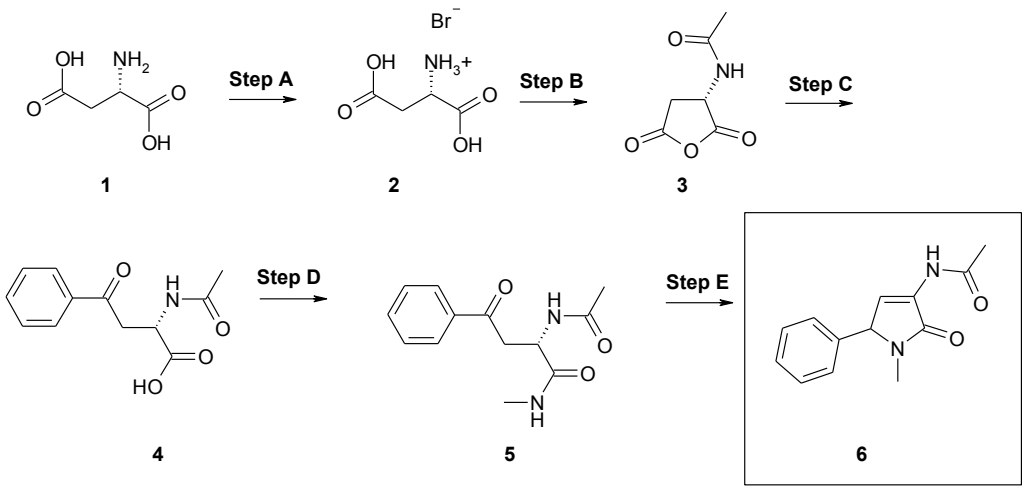
Synthesis of (1) PYR2604

Compound PYR2604 was synthesized by ENAMINE Ltd., Ukraine

1. Synthesis procedures



Step A
To a mixture of **1** (6.22 g, 46.7 mmol) and water (20 ml), 40% HBr aq. (11.34 g, 56.1 mmol) was added. Then the water was evaporated, residue was washed with MeCN (20 ml), filtered and dried to give **2** (9.5 g 95%).

Step B
A mixture of **2** (8.57g, 40.0 mmol) and Ac_2O (40.87g, 400.3 mmol) was heated at 60˚C with stirring until a clear solution was obtained. The solution was concentrated to dryness and the crystalline residue triturated with dry ether and recrystallized from Ac_2O giving **3** (3.60 g, 57%).

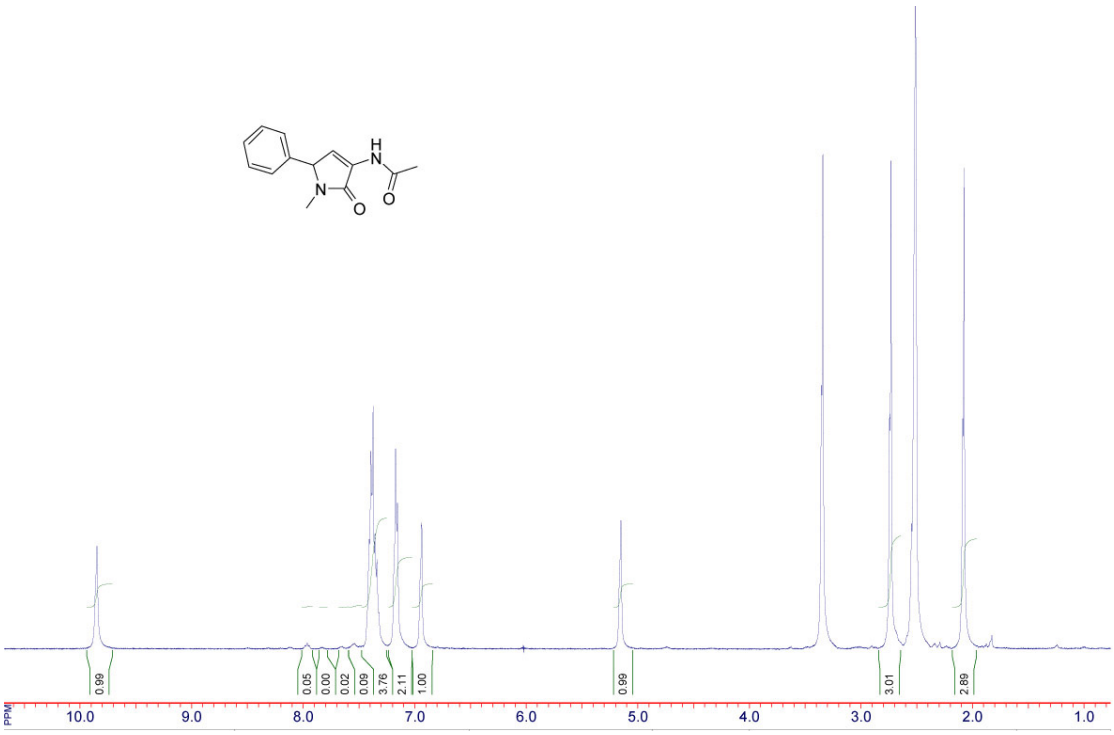
Step C
3 (2.15g, 13.7 mmol) was suspended in dry benzene (60 ml) and treated with $AlCl_3$ (5.48 g, 41.1 mmol) at 0˚C. After stirring overnight at r.t. the mixture was hydrolyzed with water (40 ml). White precipitate was collected by filtration, washed with water and dried to give **4** (2.11 g, 66%).

Step D
A mixture of **4** (0.95 g, 4.0 mmol), CDI (0.65g, 4.0 mmol), and MeCN (10 ml) was stirred at 60˚C until the gas evolution ceased. Then 20% methanolic methylamine (0.75 g, 4.8 mmol) was added. The reaction mixture was stirred for 1 hr at 60˚C. Then the mixture was concentrated to dryness and triturated with EtOAc, and the residue was recrystallized from MeCN to give amide **5** (0.32 g, 32%).

Step E
To a mixture of **5** (280 mg, 1.13 mmol) and benzene (50 ml), PTSA (20-30 mg) was added and the mixture was refluxed with water separation by a Dean-Stark trap for 1

h. The reaction mixture was then filtered, filtrate was washed with $NaHCO_3$ solution, dried with Na_2SO_4 and evaporated to dryness to give **6** (50 mg, 19%).

2. NMR



Synthesis of Dithiolopyrrolones

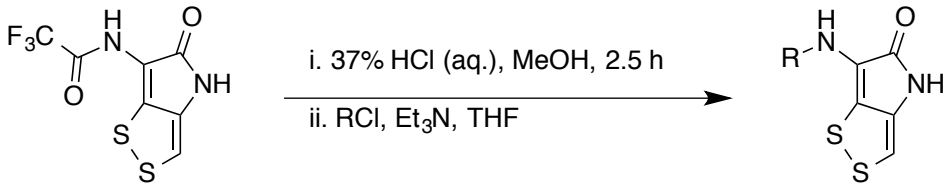
1. General Methods

All reactions were carried out in an oven-dried round-bottomed-flask under an inert nitrogen atmosphere. Solvents and reagents were used as received unless otherwise noted. Mupirocin was purchased from Applichem through Fisher scientific. Thiolutin was purchased from commercial sources. Spectra for ^{1}H and ^{13}C NMR were recorded at room temperature with a Bruker Avance^{III} (500 MHz and 125 MHz or 600 MHz and 150 MHz, respectively) or a Varian Inova 400 (400 MHz and 100 MHz, respectively). Chemical shifts are reported in δ (ppm) relative units to residual solvent peaks $CDCl_3$ (7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C) and DMSO-*d*6 (2.50 ppm for ^{1}H and 39.5 ppm for ^{13}C). Splitting patterns are assigned as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), multiplet (m), dd (doublet of doublets), and td (triplet of doublets). Mass spectrometry measurements were recorded using an

Agilent 6520 Accurate-Mass Q-TOF ESI positive in high-resolution mode. Predicted masses were extracted to ± 5 ppm.

2. Synthesis

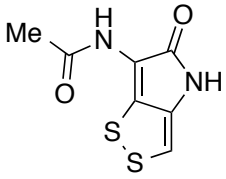
Trifluoroacetyl holothin (2,2,2-trifluoro-*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)acetamide). The compound was prepared according to the method of Hjelmgaard et al.[1] The residue was purified by flash column chromatography (Hex/EtOAc 1:2, R_f = 0.40) to obtain a yellow solid (5 steps, 13% overall yield). ^{1}H NMR (500 MHz; DMSO-d_6) δ 7.32 (s, 1H), 10.92 (s, 1H), 11.60 (s, 1H). ^{13}C NMR (125 MHz; DMSO-d_6) δ 112.0, 112.2, 115.4 (q, J = 285 Hz), 133.5, 140.3, 153.8 (q, J = 37.5 Hz), 167.5. HR-MS (ESI): Calculated $C_7H_4F_3N_2O_2S_2^+$ $[M + H]^+$ = 268.9661; found $[M + H]^+$ = 268.9664.



General procedure for the preparation of dithiolopyrrolone analogues

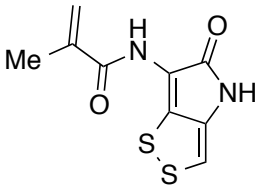
Followed a literature procedure.[1] Trifluoroacetyl holothin (0.186mmol) is dissolved in MeOH (7.25 mL) and HCl (37% aqueous, 0.24 mL) is added dropwise. Reaction is refluxed for 2.5 h. Mixture is allowed to cool to room temperature and concentrated *in vacuo* yielding a yellow-orange solid that is used without further purification (R_f = 0, Hex/EtOAC 1:2). The residual solid is suspended in THF (16.5 mL) and cooled to 0 ^{o}C. The corresponding acid chloride is added dropwise (0.231 mmol) followed by Et_3N (0.415mmol). Reaction is allowed to warm to room temperature and stirred for 30 minutes. Mixture is concentrated *in vacuo* and purified by flash column chromatography.

(2) Holomycin (*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)acetamide).



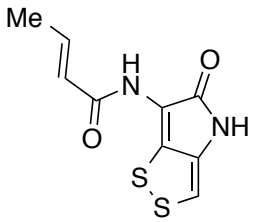
Prepared according to the general procedure. Acetyl chloride (16.4 μL, 0.231 mmol) was utilized as the acid chloride source. Flash column chromatography (R_f = 0.67, EtOAc/MeOH 9:1) yielded compound as an orange solid (57 % yield). ^{1}H NMR is in agreement with those previously reported.[1] ^{1}H NMR (500 MHz; DMSO-d_6) δ 2.00 (s, 3H), 7.04 (s, 1H), 9.89 (s, 1H), 10.71 (s, 1H). HR-MS (ESI): Calculated $[M + H]^+$ = 214.9943; found $[M + H]^+$ = 214.9946.

(3) Methacryloyl Holothin (*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)methacrylamide).



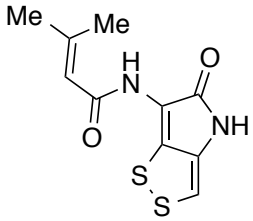
Prepared according to the general procedure. Methacryloyl chloride was utilized as the acid chloride source. Flash column chromatography (R_f = 0.15, Hex/EtOAc 1:2) yielded compound as an orange solid (39% yield). ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.92 (s, 3H), 5.50 (t, J = 1.0 Hz, 1H), 5.89 (s, 1H), 7.12 (s, 1H), 9.34 (s, 1H), 10.80 (s, 1H). ^{13}C NMR (125 MHz; DMSO-d_6) δ 18.4, 111.0, 114.9, 121.8, 133.7, 135.7, 138.1, 166.1, 168.0. HR-MS (ESI): Calculated $[M + H]^+$ = 241.0100; found $[M + H]^+$ = 241.0103.

(4) Crotonyl Holothin ((*E*)-*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)but-2-enamide).



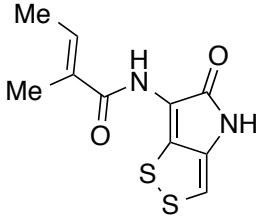
Prepared according to the general procedure. Crotonyl chloride was utilized as the acid chloride source. Flash column chromatography (R_f = 0.20, Hex/EtOAc 1:2) yielded compound as an orange solid (39% yield). ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.81 (dd, J = 1.5 and 7.0 Hz, 3H), 6.33 (dd, J = 1.5 and 15.2 Hz, 1H), 6.74 (dq, J = 6.5 and 15.5 Hz, 1H), 7.08 (s, 1H), 9.97 (s, 1H), 10.75 (s, 1H). ^{13}C NMR (125 MHz; DMSO-d_6) δ 17.7, 111.0, 115.3, 124.2, 133.8, 134.4, 163.5, 167.9. HR-MS (ESI): Calculated $[M + H]^+$ = 241.0100; found $[M + H]^+$ = 241.0103.

(5) 3,3-Dimethylacryloyl Holothin (3-methyl-*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)but-2-enamide)



Prepared according to the general procedure. 3,3-dimethylacryloyl chloride was utilized as the acid chloride source. Flash column chromatography (R_f = 0.4, Hex/EtOAc 1:2) yielded compound as an orange solid (54% yield). ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.81 (d, J = 1.0 Hz, 3H), 2.12 (d, J = 1.0 Hz, 3H), 6.07-6.09 (m, 1H), 7.04 (s, 1H), 9.73 (s, 1H), 10.72 (s, 1H). ^{13}C NMR (125 MHz; DMSO-d_6) δ 19.8, 27.2, 110.5, 115.5, 117.5, 133.7, 133.8, 152.8, 164.6, 168.0. HR-MS (ESI): Calculated $[M + H]^+$ = 255.0256; found $[M + H]^+$ = 255.0266.

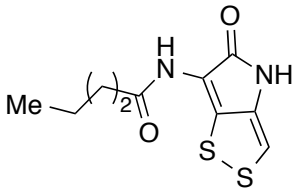
(6) Tigloyl Holothin ((*E*)-2-methyl-*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)but-2-enamide).



Prepared according to the general procedure. Tigloyl chloride was utilized as the acid chloride source. Flash column chromatography (R_f = 0.35, Hex/EtOAc 1:2) yielded compound as an orange solid (66% yield). ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.73

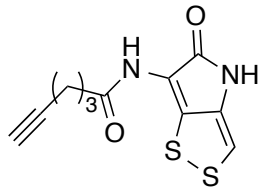
(dd, J = 1.0 and 7.0 Hz, 3H), 1.80 (t, J = 1.0 Hz, 3H), 6.53 (qq, J = 1.0 and 7.0 Hz, 1H), 7.09 (s, 1H), 9.07 (s, 1H), 10.77 (s, 1H). ^{13}C NMR (125 MHz; DMSO-d_6) δ 12.2, 13.9, 110.8, 115.3, 130.1, 132.9, 133.7, 134.9, 166.9, 168.0. HR-MS (ESI): Calculated $[M + H]^+$ = 255.0256; found $[M + H]^+$ = 255.0267.

(7) Pentanoyl Holothin (*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)pentanamide).



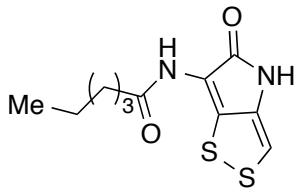
Prepared according to the general procedure. Pentanoyl chloride was utilized as the acid chloride source. Flash column chromatography (R_f = 0.10, Hex/EtOAc 1:2) yielded compound as a yellow solid (84% yield). ^{1}H NMR (500 MHz; DMSO-d_6) δ 0.86 (t, J = 7.0 Hz, 3H), 1.22-1.30 (m, 2H), 1.46-1.52 (quint, J = 7.5 Hz, 2H), 2.33 (t, J = 7.0 Hz, 2H), 7.04 (s, 1H), 9.84 (s, 1H), 10.71 (s, 1H). ^{13}C NMR (125 MHz; DMSO-d_6) δ 13.7, 21.7, 27.2, 34.4, 110.5, 115.3, 133.7, 133.9, 167.9, 171.8. HR-MS (ESI): Calculated $[M + H]^+$ = 257.0413; found $[M + H]^+$ = 257.0418.

(8) 5-Hexynoyl Holothin (*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)hex-5-ynamide).



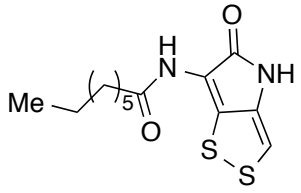
Prepared according to the general procedure. 5-hexynoyl chloride was utilized as the acid chloride source. Flash column chromatography (R_f = 0.5, Hex/EtOAc 1:2) yielded compound as a brown-orange solid (16% yield). ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.68 (quint, J = 7.0 Hz, 2H), 2.16 (td, J = 2.5 and 7.0 Hz, 2H), 2.43 (t, J = 7.0 Hz, 2H), 2.78 (t, J = 3 Hz, 1H), 7.04 (s, 1H), 9.88 (s, 1H), 10.68 (s, 1H). ^{13}C NMR (125 MHz; DMSO-d_6) δ 17.3, 24.0, 71.6, 83.9, 110.5, 115.3, 133.7, 134.1, 167.9, 171.1. HR-MS (ESI): Calculated $[M + H]^+$ = 267.0256; found $[M + H]^+$ = 267.0260.

(9) Hexanoyl Holothin (*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)hexanamide).



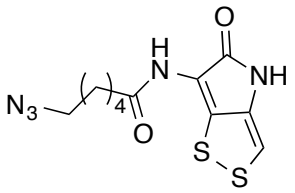
Prepared according to the general procedure. Hexanoyl chloride was utilized as the acid chloride source. Flash column chromatography (R_f = 0.60, EtOAc) yielded compound as a yellow solid (77% yield). ^{1}H NMR is in agreement with those previously reported.[1] ^{1}H NMR (500 MHz; DMSO-d_6) δ 0.85 (t, J = 7.0 Hz, 3H), 1.19-1.31 (m, 4H), 1.50 (quint, J = 7.0 Hz, 2H), 2.32 (t, J = 7.5 Hz, 2H), 7.04 (s, 1H), 9.84 (s, 1H), 10.71 (s, 1H). HR-MS (ESI): Calculated $[M + H]^+$ = 271.0569; found $[M + H]^+$ = 271.0590.

(10) Octanoyl Holothin (*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)octanamide).



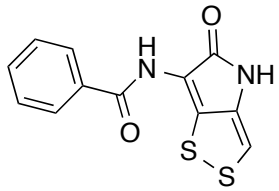
Prepared according to the general procedure. Octanoyl chloride was utilized as the acid chloride source. Flash column chromatography (R_f = 0.45, Hex/EtOAc 1:2) yielded compound as a yellow solid (77% yield). ^{1}H NMR is in agreement with those previously reported[2]. ^{1}H NMR (500 MHz; DMSO-d_6) δ 0.84 (t, J = 7.0 Hz, 3H), 1.18-1.29 (m, 8H), 1.50 (quint, J = 7.0 Hz, 2H), 2.32 (t, J = 7.5 Hz, 2H), 7.04 (s, 1H), 9.83 (s, 1H), 10.70 (s, 1H). HR-MS (ESI): Calculated $[M + H]^+$ = 299.0882; found $[M + H]^+$ = 299.0887.

(11) 6-Azidohexanoyl Holothin (6-azido-*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)hexanamide).



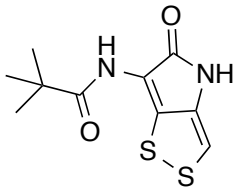
Prepared according to the general procedure. 6-azidohexanoyl chloride was utilized as the acid chloride source. Flash column chromatography (R_f = 0.3 Hex/EtOAc 1:2) yielded compound as an orange solid (19%). ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.26-1.33 (m, 2H), 1.53 (quint, J = 7.0 Hz, 4H), 2.34 (t, J = 7.5 Hz, 2H), 3.31 (t, J = 7.0 Hz, 2H), 7.05 (s, 1H), 9.86 (s, 1H), 10.71 (s). ^{13}C NMR (125 MHz; DMSO-d_6) δ 24.6, 25.7, 27.9, 34.5, 50.5, 110.6, 115.3, 133.7, 134.0, 167.9, 171.7. HR-MS (ESI): Calculated $[M + H]^+$ = 312.0583; found $[M + H]^+$ = 312.0592.

(12) Benzoyl Holothin (*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-b]pyrrol-6-yl)benzamide).



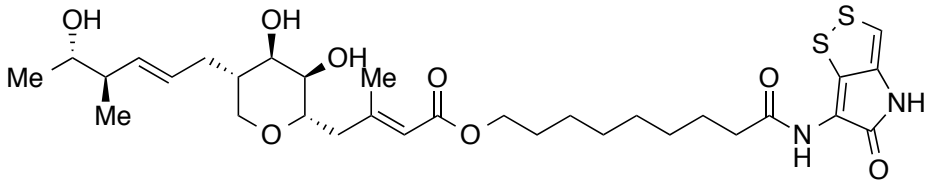
Prepared according to the general procedure. Benzoyl chloride was utilized as the acid chloride source. Flash column chromatography (R_f = 0.10, Hex/EtOAc 1:2) yielded compound as a yellow solid (72% yield). ^{1}H NMR is in agreement with those previously reported[1]. ^{1}H NMR (400 MHz; DMSO-d_6) δ 7.16 (s, 1H), 7.47-7.51 (m, 2H), 7.56-7.60 (m, 1H), 7.97-99 (m, 2H), 10.02 (s, 1H), 10.81 (s, 1H). HR-MS (ESI): Calculated $[M + H]^+$ = 277.0100; found $[M + H]^+$ = 277.0098.

(13) Pivaloyl Holothin (*tert*-Butyl (5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-b]pyrrol-6-yl)carbamate).



Prepared according to the general procedure. Pivaloyl chloride was utilized as the acid chloride source. Flash column chromatography (R_f = 0.10, Hex/EtOAc 1:2) yielded compound as a yellow solid (77% yield). ^{1}H NMR (400 MHz; DMSO-d_6) δ 1.20 (s, 9H), 7.08 (s, 1H), 8.73 (s, 1H), 10.76 (s, 1H). ^{13}C NMR (100 MHz; DMSO-d_6) δ 26.94, 110.70, 115.11, 133.61, 134.87, 168.05, 176.60. HR-MS (ESI): Calculated $[M + H]^+$ = 257.0413; found $[M + H]^+$ = 257.0405.

(14) Pseudomonyl C holothinamide (9-oxo-9-((5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)amino)nonyl (*E*)-4-((2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-((4*R*,5*S*,*E*)-5-hydroxy-4-methylhex-2-en-1-yl)tetrahydro-2*H*-pyran-2-yl)-3-methylbut-2-enoate).



Pseudomonyl C holothinamide was prepared according to a previously reported method[3]. ^{1}H NMR (600 MHz; $CDCl_3$) δ 9.17 (s, 1H), 8.01 (s, 1H), 6.88 (s, 1H), 5.76 (s, 1H), 5.45 (m, 2H), 4.07 (t, *J* = 6.3 Hz, 2H), 3.92 (t, *J* = 3.3 Hz, 1H), 3.79 (dd, *J* = 3.0, 11.4 Hz, 1H), 3.73 (td, *J* = 3.0, 9.0 Hz, 1H), 3.64 (m, 2H), 3.56 (m, 1H), 3.51 (dd, *J* = 1.8, 11.4 Hz, 1H), 3.47 (dd, *J* = 3.0, 8.4 Hz, 1H), 2.62 (dd, *J* = 2.4, 15.0 Hz, 1H), 2.44 (s, br, 3H), 2.36 (t, *J* = 7.5 Hz, 2H), 2.28 (dd, *J* = 8.1, 14.7 Hz, 1H), 2.23 (m, 1H), 2.20 (d, *J* = 0.6 Hz, 3H), 2.16 (m, 1H), 2.09 (m, 1H), 1.86 (m, 1H), 1.69 (m, 1H), 1.62 (m, 1H), 1.32 (m, 8H), 1.16 (d, *J* = 6.0 Hz, 3H), 0.99 (d, *J* = 7.2 Hz, 3H). HR-MS (ESI): Calculated $[M + H]^+$ = 639.2768; found $[M + H]^+$ = 639.2765.

References

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